Root and shoot development of *Rumex* species under waterlogged conditions

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The responses of *Rumex acetosa*, *Rumex crispus*, and *Rumex palustris* to different levels of waterlogging were studied in sand culture experiments. *Rumex crispus* and *R. palustris* developed new flooding-resistant roots as a response to waterlogging. The growth of these new roots caused a changed vertical distribution of the root length in these species; most root length was concentrated in the upper 10 cm of the soil. *Rumex acetosa* did not show significant development of flooding-resistant roots and did not change its vertical root distribution during flooding of the soil. The results of the experiments indicate that growth expressed as relative growth rate is positively correlated to the development of new flooding-resistant roots under waterlogged conditions. We concluded that *R. crispus* and *R. palustris* are more resistant to waterlogging than *R. acetosa*; this agrees with the distribution of these *Rumex* species in the field. However, waterlogging resistance is probably only one of the factors influencing differences in field location between the *Rumex* species.

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Les réponses des *Rumex acetosa*, *Rumex crispus* et *Rumex palustris* à différents niveaux d'imbibition ont été étudiées dans des expériences de culture en sable. Chez les *Rumex crispus* et *R. palustris*, il y a eu comme réaction la formation de nouvelles racines résistantes aux inondations. La croissance de ces nouvelles racines a causé un changement dans la distribution verticale de la longueur des racines chez ces espèces; la plus grande partie de la longueur radiculaire se trouvait dans les 10 centimètres supérieurs du sol. Chez le *Rumex acetosa*, il n'y avait presqu'aucune différenciation de racines résistantes aux inondations ni aucun changement dans la distribution verticale des racines au cours de l'inondation du sol. Ces résultats indiquent que la croissance exprimée en taux relatif de croissance est en corrélation positive avec le développement de nouvelles racines résistantes aux inondations sous des conditions d'imbibition du sol. On a conclu que les *R. crispus* et *R. palustris* étaient plus résistants à l'imbibition que le *R. acetosa*, ce qui est en accord avec la distribution de ces espèces du *Rumex* sur le terrain. Cependant, les différences dans la localisation sur le terrain des espèces du *Rumex* ne sont probablement pas attribuables qu'aux seuls effets de l'imbibition.

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Introduction

The river areas in The Netherlands have a strongly fluctuating water table and sometimes become flooded with river water. During the winter these floodings are more or less predictable, whereas in summer both frequency and duration of the inundations are erratic. In these areas, *Rumex* species are distributed along a flooding gradient. *Rumex acetosa* is found on high, seldomly flooded dykes and river levees; *Rumex palustris* occurs on very low, frequently inundated mudflats of former river beds. *Rumex crispus* has an intermediate position (Voesenek and Blom 1987). It is hypothesized that this zonation of *Rumex* species in the river areas is mainly determined by the flooding frequency and duration; differences in flooding resistance can be important in explaining species-specific locations in the flooding gradient. Flooding can completely submerge the plant or it can be restricted to flooding of the soil only (waterlogging). In the river areas of The Netherlands, waterlogging is a transient phase between periods in which the soil is drained and periods with complete submergence.

Waterlogging causes physical, chemical, and biological changes in the soil environment (Armstrong 1975; Ponnamperuma 1984). The low solubility and the low diffusion rate of oxygen in water are responsible for the reduced exchange of oxygen between the atmosphere and the soil (Keeley 1979). Oxygen remaining in the soil after flooding is consumed by the respiration of roots and microorganisms (Ponnamperuma 1984) and only a very small zone near the water surface may remain oxygenated (Drew 1983). A few adaptations of plants to flooding of the soil are known, e.g., the formation of aerenchymatic tissues that allow aeration of root tissue, thereby enhancing aerobic root respiration in flooded soils (Jackson and Drew 1984). Another adaptation is a change of vertical distribution of the root system during waterlogging. The growth of new roots is mainly concentrated in the upper soil layer; this causes a very superficial rooting pattern (Drew and Sisworo 1979; Jackson and Drew 1984).

Two sand culture experiments were designed to investigate the responses of the three *Rumex* species to waterlogging. The results obtained under controlled conditions are discussed in relation to the locations of the three species in the field.

Materials and methods

Plant species and field observations

Eight *Rumex* species occur in the floodplain of the Rhine River in The Netherlands. Three of them, growing in areas characterized by different inundation regimes, were selected for this study. *Rumex acetosa* L. and *R. crispus* L. are common grassland plants, not restricted to the river habitat. *Rumex palustris* Sm. is mainly found on mudflats. *Rumex acetosa* and *R. crispus* are perennials, whereas *R. palustris* is an annual or biennial. The root systems of *R. crispus* and *R. palustris* are dominated by a tap root, that of *R. acetosa* by a fibrous root pattern (Kutchera 1960; Voesenek and Blom 1987). Achenes with perianths of the three *Rumex* species were collected in 1985 in the river area. All fruits were stored in the dark at room temperature until used. Mixtures of achenes from several plants per species belonging to one river population each (*R. acetosa*: population near the village of Winssen; *R. crispus* and *R. palustris*: populations near the village of Kekerdom) were used in the experiments.

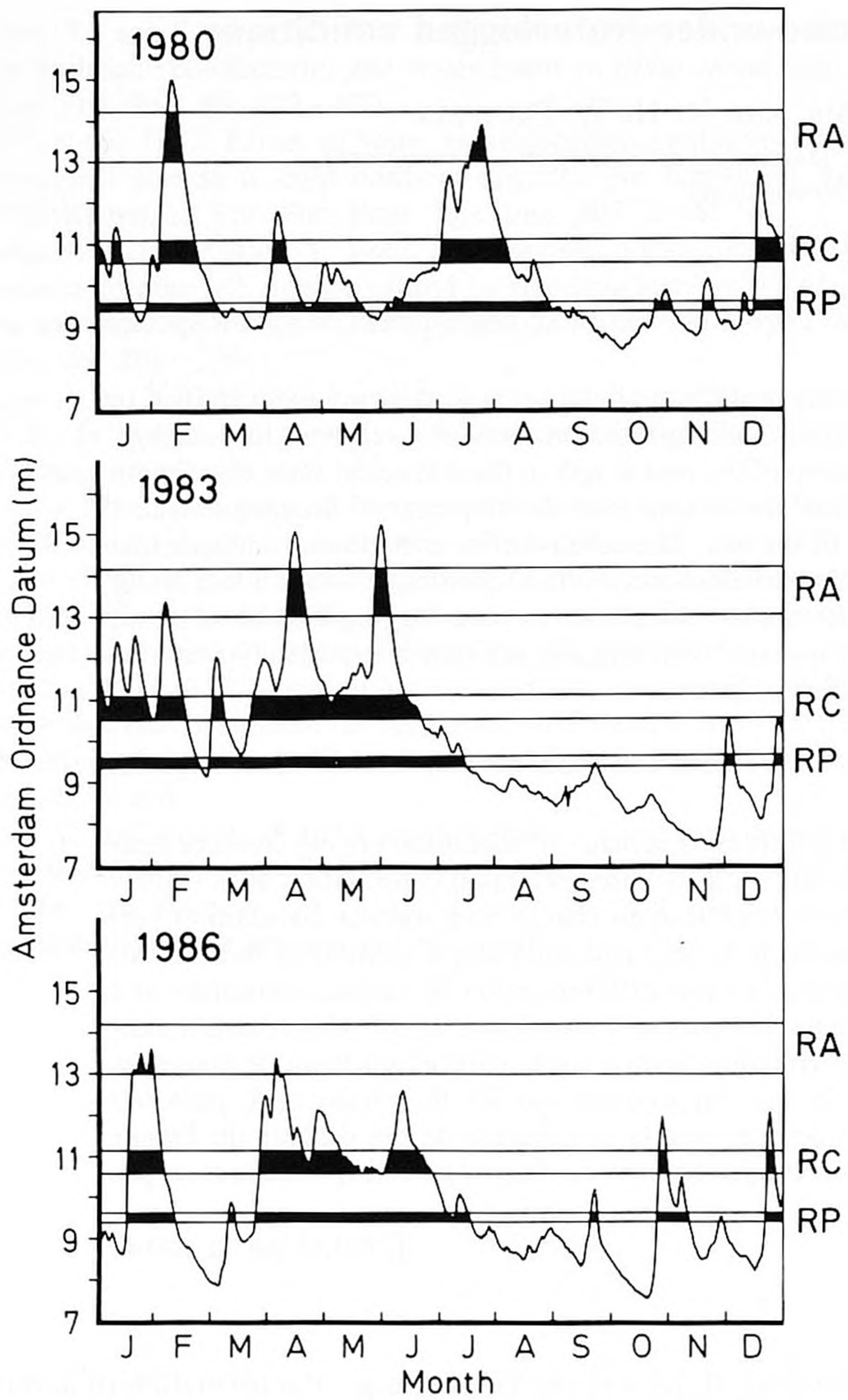

FIG. 1. The changing waterlevels of the Rhine River in the study area near Nijmegen during 1980, 1983, and 1986. The locations of three zones with different *Rumex* species are included in the diagrams. RA, *Rumex acetosa*; RC, *Rumex crispus*; RP, *Rumex palustris*.

The changing water levels of the Rhine in the study area near Nijmegen were recorded in 1980, 1983, and 1986 and are presented in Fig. 1. The positions of the three *Rumex* species were measured with a levelling instrument and are included in Fig. 1.

Experiments

A sand culture experiment in the greenhouse was designed to determine the differences in responses among the three *Rumex* species to different levels of waterlogging. *Rumex* achenes were sown in PVC jars (depth 50 cm; diameter 16 cm). Each jar contained five seeds and 30 jars were prepared for each *Rumex* species. After seedling emergence, the number of plants per jar was randomly reduced to one. The soil in the jars was regularly watered to keep the soil moisture near field capacity. Twice a week they were watered with 0.5 strength Hoagland's solution. Seven weeks after sowing, the plants were exposed to different water regimes. Nine jars per species were placed in containers with 0.1 strength Hoagland's solution 25 cm deep (partial waterlogging (PW)); another nine jars were placed in containers with 0.1 strength Hoagland's solution 50 cm deep, (waterlogging (W)). A drained water regime was maintained in the 12 remaining jars per species (control (C)). The water levels in the containers were kept constant. On day 0, three plants per species were harvested from control treatments. On days 12, 26, and 40 after starting the treatments, three plants per species per treatment were harvested. Waterlogging is mostly a short, transient phase in the river areas. The fast rise and sharp decline of the river water levels (Fig. 1), related to the rapid runoff of rainwater from the upper reaches of the Rhine, restrict the duration of soil flooding; therefore, the treatments were continued for a maximum of 40 days. At harvest the soil monoliths were washed out on a pinboard. The distance between the steel pins was 2.0 cm and the length of each pin was 8.5 cm. After washing, the total root system was divided into five vertical layers of 10 cm each. Dry weight (72 h at 80°C) of shoots and of roots per layer, and root length per layer (Comair root length scanner (Rowse and Phillips 1974)) were determined. Leaf area, number of leaves, and the length of the largest leaf were also measured. Mean relative growth rates (RGR) were calculated according to the formula first described by Fisher (1921).

In a separate experiment in the greenhouse, the length of new roots developed after waterlogging was determined. For this experiment, *Rumex* achenes were sown in pots with a depth of 13 cm and a diameter of 13 cm. The large 50-cm pots were not used in this experiment because data concerning the vertical distribution of the roots were not collected. After seedling emergence, the number of plants per pot was randomly reduced to one. Six weeks after sowing, the plants were placed in containers with 0.1 strength Hoagland's solution 13 cm deep (waterlogging treatment). On days 7, 14, 21, and 35 after starting the waterlogging treatment, four plants of *R. acetosa*, five plants each of *R. crispus*, and *R. palustris* were harvested. At these harvests, the length of new roots produced during the treatment was measured. These new roots were distinguished from older ones by the differences in colour and diameter.

During both experiments, the temperature in the greenhouse varied from 15°C dark to 20–30°C light (16-h photoperiod). The plants were illuminated by supplementary high-pressure sodium lamps (400 W) with a photon flux density at plant level of 115 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Statistical analysis

The root and shoot data and the root:shoot ratio (RSR) were analysed using a two-way analysis of variance (ANOVA), with species, treatments, and their interaction as sources of variance (root length was analysed after $\log_e$ transformation). The RGR calculated over several treatment periods was statistically analysed using a two-way ANOVA, with species, treatments, and their interaction as sources of variance. The root length (percentage after angular transformation) of each soil layer was analysed for each harvest using a two-way ANOVA, with species, treatments, and their interaction as sources of variance. All statistical analyses were performed using the SAS statistical package (SAS Institute Inc. 1985).

Results

The statistical analysis of RGR is summarized in Table 1. The main effect of the treatment was significant in the first treatment period (0–12 days). Waterlogging and, to a lesser extent, partial waterlogging resulted in all species having lower RGR values (Fig. 2A). During the last treatment period (26–40 days), the RGR showed a significant interaction. This interaction can be explained by the reduced RGR of *R. acetosa* during the waterlogging treatment (Fig. 2C).

The statistical analysis of the RSR data after 40 days of treatment is also given in Table 1. The main effects were significant; both treatments caused a very large reduction of the RSR. The significant interaction is related to the high values in the control series of *R. crispus* and *R. palustris* (Fig. 2J).

The root systems of the three *Rumex* species exposed to the treatments were morphologically and structurally different. All original roots stopped growing and gradually died in the inundated zone of the soil. New roots developed on the tap roots of *R. crispus* and *R. palustris* within 7 days. These roots can be divided into two morphological types: (*i*) strongly

TABLE 1. Effect of waterlogging on growth during three successive treatment periods and root and shoot data after 40 days of waterlogging of *Rumex acetosa*, *R. crispus*, and *R. palustris*

	Independent variables		
Dependent variable	Species	Treatments	Interactions
RGR			
0–12 days	***	***	ns
12–26 days	ns	ns	ns
26–40 days	ns	ns	*
Root/shoot ratio	***	***	**
Root dry weight	***	***	ns
Root length[a]	***	***	***
Shoot dry weight	ns	***	***
Leaf area	*	***	**
No. of leaves	***	***	**
Largest leaf length	***	***	***

NOTE: Results of two-way ANOVA with species, treatments, and their interaction as sources of variance. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[a]Analysis was performed after $\log_e$ transformation.

TABLE 2. The length (mean ± SE) of new roots of *R. acetosa*, *R. crispus*, and *R. palustris* produced during a waterlogging treatment

	Days after waterlogging			
	7	14	21	35
R. acetosa (n=4)	0.1±0.1	0.4±0.1	0.3±0.1	0.4±0.1
R. crispus (n=5)	1.8±0.2	3.1±0.4	15.9±1.1	32.7±6.9
R. palustris (n=5)	2.3±0.4	3.2±0.3	15.0±3.0	19.1±4.6

TABLE 3. Effect of 40 days of waterlogging treatment on the relative root length (%) of *Rumex acetosa*, *R. crispus*, and *R. palustris* per 10 cm of soil layer

	Independent variables		
Depth (cm)	Species	Treatment	Interaction
0–10	***	***	***
10–20	*	***	*
20–30	**	**	***
30–40	ns	***	***
40–50	***	***	***

NOTE: Results of two-way ANOVA with species, treatments, and their interaction as sources of variance. Analysis was performed after angular transformation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

branched, thin, superficially growing roots (only in the W treatment); and (*ii*) thick, white, poorly branched roots, which penetrate into the deeper waterlogged soil layers (in both treatments). Both types were very poorly developed in *R. acetosa*. The lengths of all new roots developed during waterlogging (both types together) are presented in Table 2 (experiment 2).

The statistical analysis of root data after 40 days of treatment is summarized in Table 1. The main effects (species and treatment) are significant for both root dry weight and total root length (old and new roots together). Significant interactions occur only for length (Fig. 2E). The W treatment leads to a reduction of root dry weight (main effect) of all *Rumex* species under study (Fig. 2D). The total root length of *R. acetosa* after the W treatment was relatively low (Fig. 2E). The treatments influenced not only the total dry weight and length of the root systems but also the vertical distribution of the roots. The statistical analysis of the total root length (%) per soil layer after 40 days of treatment is given in Table 3. Nearly all main effects and interactions in all soil layers were significant. An increase of the relative root length (percentage of length in all layers) after W treatment in the uppermost soil layer was observed in *R. crispus* and *R. palustris*, whereas *R. acetosa* lacked this increase. In *R. crispus* and *R. palustris*, the W treatment resulted in a reduced relative root length in the deeper soil layers (20–50 cm), whereas *R. acetosa* only showed slight changes in these layers after the same treatment. Only the deepest soil layer (40–50 cm) of *R. acetosa* contained a reduced relative root length. The PW treatment resulted in a concentration of root length in the nonwaterlogged part of the soil monolith (0–25 cm) and a reduction of length in the waterlogged part (25–50 cm) in all species (Fig. 3).

The statistical analysis of the shoot dry weight after 40 days of treatment is summarized in Table 1. Treatments and interactions were significant for the shoot dry weight. The three *Rumex* species showed a higher dry weight of the shoot after 40 days of PW treatment. The interaction term can be explained by the low values of the *R. crispus* and *R. palustris* controls (Fig. 2F). *Rumex acetosa* showed a relatively low leaf area after 40 days of W treatment (Fig. 2G). This lower leaf area is not only caused by a lower number of leaves (Fig. 2H), but must also be explained by a reduced increase of leaf length during the W treatment (Fig. 2I). The W treatment resulted in an increase in leaf length of the largest leaf after 40 days in both *R. crispus* and *R. palustris*.

Discussion

All *Rumex* species under study showed reduced growth during the first 12 days of waterlogging. A relationship between this reduced growth rate and the restricted development of new flooding-resistant roots is likely. The functional significance of these new roots is accentuated by the continued low growth rate of *R. acetosa* and the very restricted development of new roots in this species during the 26- to 40-day period of the waterlogging treatment. In contrast, *R. crispus* and *R. palustris* showed a considerable development of new flooding-resistant roots and a "recovered" growth rate during this period.

All *Rumex* species under study showed decreases in RSR after both waterlogging treatments. A reduction of RSR is commonly observed after waterlogging (Sena Gomes and Kozlowski 1980; Kozlowski 1984), even in flood-tolerant species (Keeley 1979). The strongest decrease of RSR was observed in the tap root species *R. crispus* and *R. palustris*. During PW and W treatments, the development of the tap root was strongly reduced. In the partial waterlogging treatment, a thick tap root was only developed in the well-aerated zone of the soil; its development ceased abruptly as soon as the water level was reached.

The strongly branched new roots, mainly developed in the tap-root species, showed a tendency to horizontal growth. The observed diageotropism in this root type enables close contact of the roots with the air–water interface where sufficient dissolved oxygen is present to support growth and nutrient uptake (e.g., Jackson and Drew 1984).

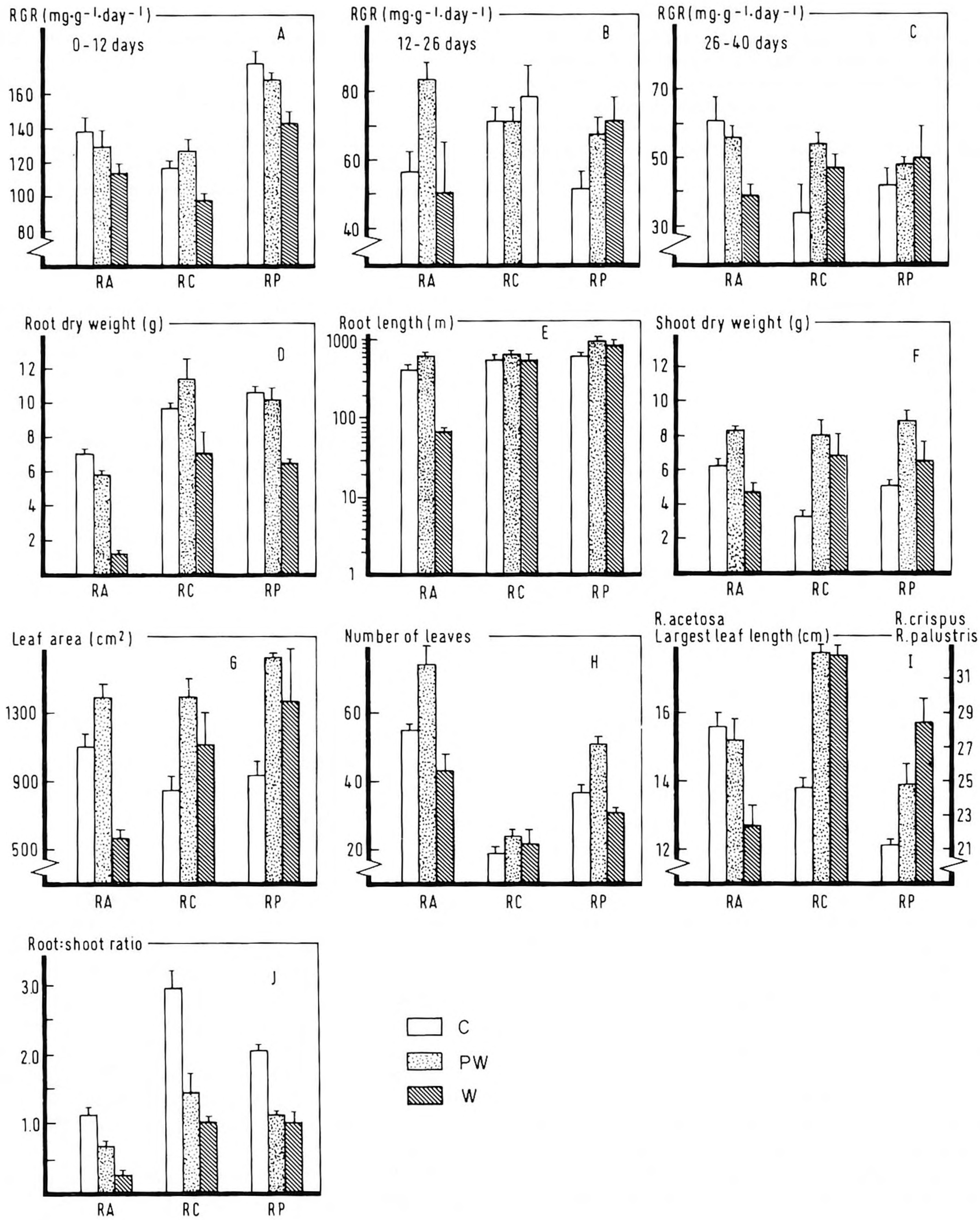

FIG. 2. (A−C) Growth (expressed as RGR + 1 SE; $n = 3$) of three *Rumex* species during three successive periods with waterlogging treatments. (D−J) Root and shoot data after 40 days of waterlogging treatments. RA, *Rumex acetosa*; RC, *Rumex crispus*; RP, *Rumex palustris*; C, control; PW, partial waterlogging; W, waterlogging.

Both *R. crispus* and *R. palustris* showed a marked change in the vertical distribution of the root length during the two waterlogging treatments. After W treatment, most of the root length of these tap root species was concentrated in the top 10 cm. This change in vertical distribution of root length was caused by a strong development of new roots on the tap root in the upper soil layer. Several authors have described similar changes in vertical distribution of roots after waterlogging (Drew and Sisworo 1979; Etherington 1983; Seliskar 1983; Jackson and Drew 1984; Waldren et al. 1987). The limited changes in the vertical distribution of root length of *R. acetosa* after W treatment proves again that this species does not show significant development of new roots after waterlogging.

The results of the greenhouse experiments must be related to the distribution of the species in the field. It can be seen from the recordings of the changing water levels of the Rhine (Fig. 1) that the *R. palustris* and *R. crispus* zones are frequently flooded and the *R. acetosa* zone is rarely flooded. These data and other field observations support the view that *R. palustris* and *R. crispus* are growing more frequently under

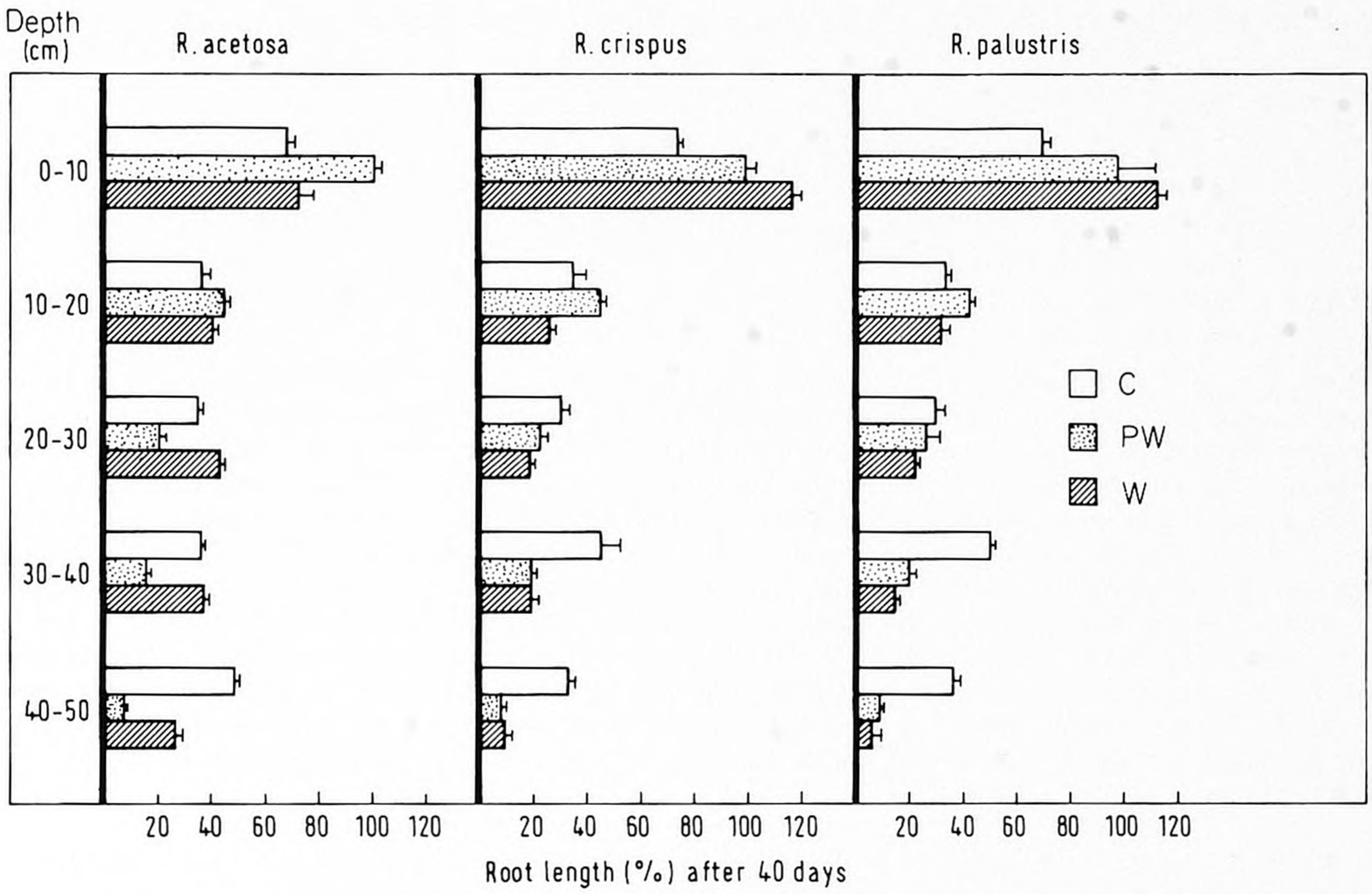

FIG. 3. Mean relative root length (% transformed to angle; + 1 SE; n = 3) per soil layer of *Rumex* species after 40 days of waterlogging treatments. C, control; PW, partial waterlogging; W, waterlogging.

waterlogged conditions than *R. acetosa*. The three *Rumex* species showed differential responses to the waterlogging treatments in the greenhouse experiments. *Rumex crispus* and *R. palustris* were able to develop a new root system as a response to flooding of the soil and seemed more resistant to waterlogging. *Rumex acetosa* was less resistant to waterlogging; it did not show significant development of new flooding-resistant roots and it showed a very restricted overall growth under these conditions. It can be concluded that the degree of waterlogging resistance of the species corresponds with their field distribution. However, results of these greenhouse experiments indicate that differences in occurrence between *R. crispus* and *R. palustris* in the field cannot be directly related to differences in waterlogging resistance. All *Rumex* species under study did survive a waterlogging period of 40 days. Therefore it is likely that waterlogging resistance is not the only factor determining the zonation of these species in the field. This emphasizes the importance of studying the effects of submergence on survival and growth under greenhouse and field conditions before definite conclusions can be drawn.

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